American Journal of Hematology 52:155–164 (1996)

Granulocytes From Chronic Myeloid Leukemia (CML) Patients Show Differential Response to Different Chemoattractants

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Binding of chemoattractant to polymorphonuclear leukocytes (PMNL) triggers a series of events like polymerization of actin and tubulin, orientation of cells, chemotaxis, increase in fluid pinocytosis and phagocytosis, and stimulation of microbicidal pathways which includes lysosomal degranulation and generation of reactive oxygen species. Earlier studies from our laboratory have shown that stimulation of chemotaxis, fluid pinocytosis, and actin polymerization of CML PMNL in response to a synthetic chemotactic peptide formyl-methionyl-leucyl-phenylalanine (fMLP) is significantly lower than that in normal PMNL. It is not known whether this lower response of CML PMNL to fMLP is a global phenomenon involving different chemoattractant receptors or is restricted to the fMLP pathway. We have evaluated chemoattractant induced degranulation process in normal and CML PMNL to fMLP, platelet activating factor (PAF), leukotriene B_4 (LTB_4), and an analogue of fMLP viz formyl-methionine-1aminocyclooctane 1 carboxylic acid-phenylalanine-O-methionine ($FACC^8$) using release of lysozyme as a parameter. We find that after stimulation with fMLP and $FACC^8$, the mean percent release of lysozyme was significantly lower in CML PMNL as compared to that in normal cells ($P < 0.001$). There was no significant difference between the two after stimulation with PAF and LTB_4. The results indicate that the fMLP pathway is suppressed in CML granulocytes whereas PAF and LTB_4 pathways appear unaltered in these cells. We therefore also studied the kinetics of peptide-receptor interaction with a labelled hexapeptide fNLPNTL which binds to the fMLP receptor. Our results show that the number of fMLP receptors/cell is significantly lower in CML PMNL ($P < 0.05$) than in normal PMNL, while their affinity constants and dissociation constants were comparable. © 1996 Wiley-Liss, Inc.

Key words: CML, granulocytes, chemoattractants, degranulation, fMLP receptors

INTRODUCTION

Polymorphonuclear leukocytes (PMNL) play an important role in the host inflammatory response. These cells remotely sense infections due to their ability to detect chemoattractants made by microorganisms or formed in the inflammatory process. Binding of chemoattractant to receptors on neutrophil surface ultimately results in uptake and killing of microorganisms. A series of events which are stimulated by binding of chemotactic compounds to the surface of these cells are: polymerization of actin and tubulin, polarization of cells, chemotaxis, pinocytosis and phagocytosis, and stimulation of microbicidal pathways. These include release of lysosomal enzymes, degranulation, generation of various reactive oxygen species, etc. [1,2].

Chronic myeloid leukemia (CML) is a clonal disorder characterized by the presence of Philadelphia chromosome in the myeloid cells [3,4]. Polymorphonuclear leukocytes (PMNL) from CML patients are morphologically indistinguishable from normal PMNL. Previous studies

Received for publication December 20, 1994; accepted January 17, 1996.

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from our laboratory have shown that chemotaxis, stimulated by chemotactic peptide n-formyl-methionyl-leucyl-phenylalanine (fMLP), is suppressed in CML PMNL [5,6]. This defect in chemotaxis may be due to alterations in fMLP-receptor interaction, in the signal transduction system, or due to alterations in the regulation of cytoskeletal proteins responsible for motility. Actin microfilaments (MF) form the motile apparatus in PMNL. We have earlier shown that fluid pinocytosis and polymerisation of actin stimulated by fMLP is significantly lower in CML PMNL [7–9]. Chemotaxis is also stimulated by compounds such as platelet activating factor (PAF), leukotriene B_4 (LTB_4), complement factor 5a (C5a), neutrophil activating peptide (NAP), etc., which act through independent receptors [10]. It is not known whether the lower response of CML PMNL to fMLP is a part of a global phenomenon involving all the chemoattractant receptors or it is restricted only to the fMLP pathway. We have studied the response of normal and CML PMNL to fMLP, PAF, LTB_4, and an analogue of fMLP, namely, formyl-methionyl-1-amino-cyclooctane-1-carboxylic acid-phenylalanine-Omethionine ($FACC^8$), using release of lysozyme as an end point parameter. We find that stimulation by fMLP pathway is significantly reduced in CML PMNL. Hence, kinetics of fMLP receptor interaction were studied in normal and CML PMNL using the hexapeptide formyl-norleucyl-leucyl-phenylalanyl-norleucyl-tyrrosinyl-lysine (fNLPNTL), which has been shown to bind to the receptor for the chemotactic peptide fMLP [11]. We find that the number of the receptors is significantly reduced in CML PMNL as compared to normal PMNL but there is no significant difference in the binding affinity of the ligand between the two cell types.

MATERIALS AND METHODS

Chemicals

fMLP, PAF, LTB_4, sodium ditrizoate (Hypaque), Micrococcus lysodiekticus, egg white Lysozyme, and Triton X-100 and bovine serum albumin (BSA) were obtained from Sigma Chemical Co. (St. Louis, MO). Ficoll was obtained from Pharmacia Fine Chemical Co. (Uppsala, Sweden). $FACC^8$ was a gift from Prof. P. Balram, Indian Institute of Science, Bangalore, India. ^{125}I was obtained from Amersham (Buckinghamshire, U.K.). Sep-pak C18 cartridges were obtained from Millipore Corporation (Bedford, MA). fNLPNTL was synthesized in the laboratory.

Patients were diagnosed for CML on the basis of standard clinical and hematological criteria. Peripheral blood was collected from CML patients at the time of diagnosis before commencement of therapy. Blood samples from normal individuals were used as controls. PMNL from heparinized peripheral blood were isolated on a ficoll-hypaque gradient [11] and residual red blood cells were removed by hypotonic lysis as described earlier [9]. The PMNL were suspended in phosphate buffered saline containing calcium and glucose (PBS). The cells were stained with Giemsa to estimate their enrichment. Samples with greater than 90% enrichment of PMNL and with viability more than 95% as tested by dye exclusion using 0.4% erythrosin B were used for assays. (The cells were stained with Giemsa to estimate their enrichment. Samples with greater than 90% enrichment of PMNL and with viability more than 95% as tested by dye exclusion using 0.4% erythrosin B were used for assays.)

Stock solutions of chemoattractants were prepared as follows: fMLP-10^{-3} M in dimethyl sulfoxide (DMSO), $FACC^8$-10^{-3} M in DMSO, PAF-10^{-4} M in chloroform (CHCL3), LTB_4-10^{-4} M in ethanol (EtOH). The effect of chemoattractants on degranulation of PMNL was determined using lysozyme as marker. PMNL 1×10^7 cells/ml were preincubated in a shaker water bath at 37°C for 10 min. The chemoattractants were added at their optimum concentrations and incubated for 15 min. The optimum concentrations of stimulants and the optimum time of stimulation were determined experimentally. The reaction was terminated by placing the tubes in ice. The cells were spun at 4,000 rpm for 2 min. The supernatants were collected and aliquotes were taken for estimation of lysozyme. The cells were lysed in PBS containing 0.5% Triton X-100 to measure the residual lysozyme activity. Lysozyme activity of the supernatants and the pellet was determined by measuring the rate of lysis of micrococcus lysodiekticus using a turbidometric index [13,14]. Supernatant and the cell lysate (0.3 ml each) were added to 2.7 ml of substrate (3 mg/10 ml). After rapid mixing, the change in absorbance at 450 nm was measured over a period of 3 min at 30-sec-intervals using buffer as reference in a Shimadzu UV 160A spectrophotometer at 450 nm. The results are expressed as ratio of lysozyme released in supernatants of stimulated cells to that in unstimulated solvent controls. The total lysozyme activity was expressed as the sum of the activity released in supernatants in response to chemoattractant and the residual activity present in the pellet. The enzyme secreted in the supernatant was expressed as percentage of the total lysozyme content in neutrophils referred to hereafter as percent enzyme released (PER). Solvent controls consisted of cells treated with an appropriate concentration of the respective solvent alone and temperature controls consisted of cells incubated at 37° and 4°C without chemoattractants.

Statistical Analysis

Non-parametric tests were applied for statistical analysis of the data. Signed test for paired samples and Wilcoxon signed rank test were used to compare between the percent release of lysozyme under stimulated and unstimulated conditions in PMNL from normal subjects and CML patients. The Mann-Whitney Wilcoxon test was

used to compare the percent release of enzyme in PMNL from normal subjects with that of CML patients and also to compare the ratio of lysozyme released in stimulated condition to unstimulated ones in PMNL from normal subjects with that of CML patients. PER for each group of samples is expressed as mean ± standard error of the mean (SEM).

Synthesis of fNLPNTL

The peptide was synthesized on LKB Biolynx 4175 peptide synthesizer using fluorenyl methoxy carbonyl chemistry [15]. Formylation was carried out when the peptide was attached to the resin. The peptide was cleaved from the solid support by trifluoroacetic acid:thioanisol:metacresol:ethenedithiol (10:1:1:1 v/v). The peptide was checked for purity by reverse phase FPLC and further characterized by amino acid analysis.

Iodination of fNLPNTL

Iodination of fNLPNTL was done with Chloramine T according to Niedel et al. [11] with some modifications. The labelled peptide mixture was passed through Sep-pak C18 affinity cartridge to bind the labelled peptide. The column was washed with 10 ml of 25 mM Kl followed by 10 ml of double distilled water to remove unlabelled iodine. The sep-pak C18 cartridge was then inverted and the iodinated peptide was eluted with 2 ml each of 25 and 50% acetonitrile containing 0.1% TFA. One milliliter fractions were collected and 10 μl aliquot from each fraction was counted in a γ counter (Nuclear Enterprises, Ltd., Edinburgh, UK). Fractions with high counts were pooled and used for binding assay. The labelling efficiency or percent incorporation ranged from 65.3 to 77.1%. The specific activity of the peptide ranged from 158.3 μci/μg of peptide to 192.8 μci/μg of peptide.

Biological activity of the unlabelled and the labelled peptide was checked by studying degranulation using lysozyme as a marker enzyme [9]. PMNL from 3 normal subjects were simulated using varying concentrations (10^{-5}–10^{-7}M) of labelled and unlabelled peptide. Maximum enzyme release was obtained for both the peptides at a concentration of 10^{-6}M (data not shown). This enzyme release was similar to that obtained in cells stimulated with 10^{-6}M fMLP.

Non-specific binding, determined by competition experiments, was estimated as the amount of labelled peptide bound to the PMNL in the presence of 1×10^{-4}M of unlabelled peptide (fNLPNTL).

Binding assay was carried out in plasic tubes coated with 1% BSA. PMNL (1×10^7 cells/ml) were suspended in PBS containing 1.0 mM Ca^{++} and 0.5% BSA. One hundred microliters of the suspension was incubated at 24°C for 30 min with equal volume of varying concentrations of ^{125}I-fNLPNTL ranging from 300×10^{-10}–9.375×10^{-10}M. The reaction was terminated by the addition of 2 ml precooled 1 × PBS followed by centrifugation at 1,000 rpm for 10 min. The supernatant was discarded and the cell pellet was washed with 2 ml 1 × PBS. The incorporation of the radioligand in the pellet was measured in a Gamma Counter which offered identical counting efficiency in all the 12 channels and provided background correction facilities. Specific binding was determined by subtracting the amount of radioactive ligand bound to the cells in the presence of unlabelled fNLPNTL from the amount of radioactive ligand bound in the absence of competitor. The amount of bound ligand and bound/free ligand (B/F) was calculated and plotted against each other. The slope obtained was used to calculate affinity constant (k_a) and the X intercept was used to calculate the number of receptors [10].

The number of receptors/PMNL, affinity constants, and dissociation constants showed a skewed distribution in PMNL from normal and CML samples. Therefore, a non-parametric test was applied for statistical analysis of the data. The Mann-Whitney Wilcoxon test was used to compare the number of receptors per PMNL, affinity constants and dissociation constants in PMNL from normal subjects with that of CML patients. Affinity constants, dissociation constants, and number of receptors are expressed as mean ± standard error of the mean (SEM).

RESULTS

The PMNL were stimulated with concentrations of chemoattractants varying from 10^{-5} to 10^{-8}M and incubated for 5, 10, 15, and 30 min to determine the optimum concentration and incubation period. Cells from eight different normal subjects and five different CML patients each were used in these assays for each chemoattractant independently. The optimum concentration for fMLP and fACC[8] was 10^{-6}M for normal and 10^{-5}M for CML cells while that for PAF and LTB_4 was 10^{-6}M for both normal and CML cells (data not shown). The optimal incubation time for the release of lysozyme was 15 min for all four chemoattractants used.

The mean PER in PMNL from 28 normal subjects under control conditions was 12.18 ± 1.13 (DMSO), 11.87 ± 1.32 (37°C), and 6.57 ± 1.13 (4°C), respectively, while that after stimulation with fMLP was 28.41 ± 1.38 (Fig. 1A). The latter is significantly higher than its solvent control ($P < 0.001$). The mean PER for cells from 23 CML patients under control conditions was 12.96 ± 1.37 (DMSO), 8.55 ± 1.5 (37°C), and 7.25 ± 1.77 (4°C), respectively, while that after stimulation with fMLP was 18.41 ± 1.75 (Fig. 1B). Again, there was significant increase in the PER after stimulation with fMLP over the solvent control ($P < 0.05$). The comparison between the mean PER value of CML and the normal PMNL after stimulation with fMLP showed that the latter is significantly higher than the former ($P < 0.001$). In cells stimu-

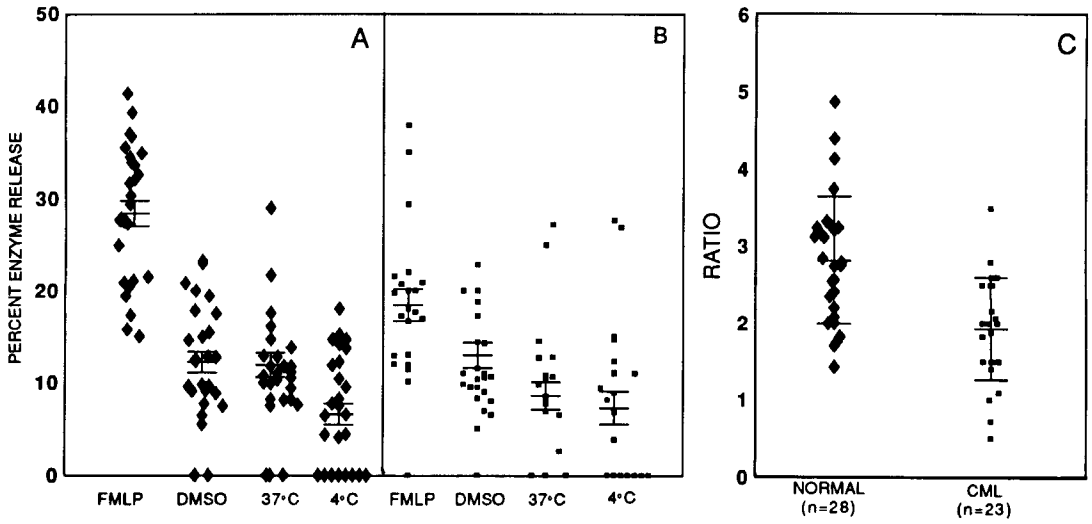

Fig. 1. Scattergram showing PER by cells stimulated with fMLP and under control conditions (DMSO, 37°, 4°C). Ordinate: PER: A: Normal PMNL. B: CML PMNL. C: Ratio of lysozyme released after stimulation with fMLP as compared to that under unstimulated conditions in normal and CML PMNL. Bars show mean ± SEM.

lated with FMLP, the ratio of lysozyme released under stimulated conditions as compared to that under unstimulated conditions was 2.82 ± 0.83 in 28 normal subjects and 1.93 ± 0.67 in 23 CML patients (Fig. 1C). The ratio is significantly higher in normal PMNL as compared to that of CML PMNL ($P < 0.05$).

The mean PER in PMNL from 22 normal subjects under control conditions was 9.41 ± 0.99 (DMSO), 11.16 ± 1.09 (37°C), and 6.91 ± 0.91 (4°C), respectively, while that after stimulation with FACC[8] was 26.56 ± 1.42 (Fig. 2A). There was a significant increase in the mean PER after stimulation with FACC[8] over its solvent control ($P < 0.001$). The mean PER in PMNL from 21 CML patients under control conditions was 11.33 ± 1.38 (DMSO), 5.91 ± 1.19 (37°C), and 3.49 ± 0.89 (4°C), respectively, while that after stimulation with FACC[8] was 16.22 ± 0.98 (Fig. 2B). The mean PER after stimulation with FACC[8] was significantly higher as compared to that in solvent control ($P < 0.05$). The comparison between the mean PER values of CML and the normal PMNL after stimulation with FACC[8] showed that the mean PER for normal PMNL is significantly higher than that for CML PMNL ($P < 0.001$). In cells stimulated with FACC[8], the ratio of lysozyme released under stimulated conditions as compared to that under unstimulated conditions was 3.23 ± 0.257 in 22 normal subjects and 1.48 ± 0.125 in 21 CML patients (Fig. 2C). The ratio is significantly higher in normal PMNL as compared to that of CML PMNL ($P < 0.001$).

When PMNL from 12 normal subjects were stimulated with PAF, the mean PER was 34.46 ± 1.35 while that in controls was 9.46 ± 2.15 ($CHCl_3$), 8.47 ± 1.98 (37°C), and 7.88 ± 1.81 (4°C), respectively (Fig. 3A). This was significantly higher than that in solvent controls ($P < 0.005$). The mean PER in PMNL from 11 CML patients under control conditions was 12.24 ± 2.36 ($CHCl_3$), 7.79 ± 1.83 (37°C), and 6.23 ± 1.88 (4°C), respectively, while that after stimulation with PAF was 29.95 ± 2.56 (Fig. 3B). There was significant increase in the mean PER after stimulation over the control conditions ($P < 0.005$). The mean PER values of CML and the normal PMNL after stimulation with PAF did not differ significantly. In cells stimulated with PAF, the ratio of lysozyme released under stimulated conditions as compared to that under unstimulated conditions was 3.40 ± 0.23 in 12 normal subjects and 3.37 ± 0.39 in 11 CML patients (Fig. 3C), which were not significantly different.

The mean PER in PMNL from 10 normal subjects under control conditions was 9.85 ± 2.08 (EtOH), 11.11 ± 1.85 (37°C), and 8.31 ± 1.31 (4°C), respectively, while that after stimulation with LTB_4 was 24.86 ± 2.08 (Fig. 4A). The mean PER after stimulation with LTB_4 was significantly higher as compared to that under control conditions ($P < 0.005$). The mean PER in PMNL from 12 CML patients under control conditions was 9.33 ± 1.68 (EtOH), 4.36 ± 1.32 (37°C), and 5.00 ± 1.63 (4°C) while that after stimulation with LTB_4 was 23.08 ± 2.12 (Fig. 4B). Again, there was a significant increase in the mean PER after stimulation with LTB_4 over the control conditions ($P < 0.005$). A comparison between the mean PER values of CML and normal PMNL showed no significant difference after stimulation with LTB_4. In cells stimulated with LTB_4 the ratio of lysozyme released under stimulated condition as compared to that under unstimulated condition was 2.67 ± 0.21 in 10 normal subjects and 2.81 ±

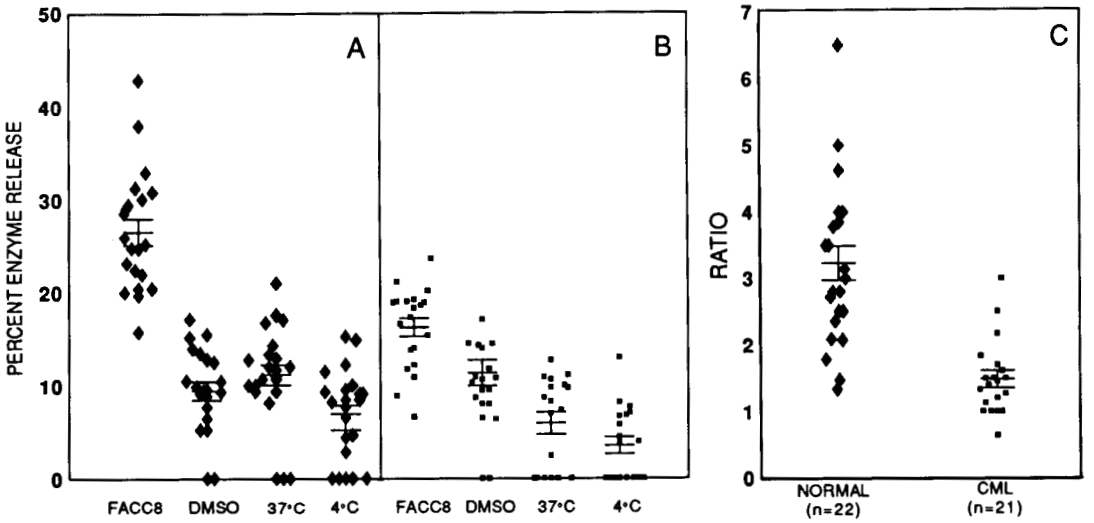

Fig. 2. Scattergram showing PER by cells stimulated with FACC[8] and under control conditions (DMSO, 37°, 4°C). Ordinate: PER: A: Normal PMNL. B: CML PMNL. C: Ratio of lysozyme released after stimulation with FACC[8] as compared to that under unstimulated conditions in normal and CML PMNL. Bars show mean ± SEM.

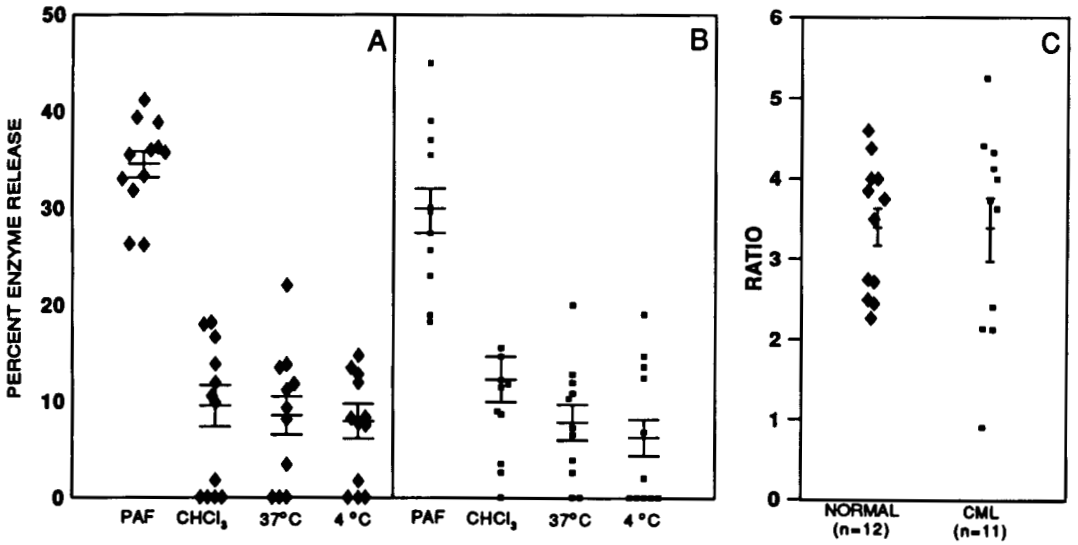

Fig. 3. Scattergram showing PER by cells stimulated with PAF and under control conditions ($CHCl_3$, 37°, 4°C). Ordinate: PER: A: Normal PMNL. B: CML PMNL. C: Ratio of lysozyme released after stimulation with PAF as compared to that under unstimulated conditions in normal and CML PMNL. Bars show mean ± SEM.

0.24 in CML patients (Fig. 4C). There was no significant difference between these values.

Analysis of fMLP Receptors With fNLPNTL

In the analysis of ligand-receptor interactions involving low and high affinity receptors, the proportion of bound receptors plotted against the total number of receptors yields a biphasic graph where the first downward slope indicates high affinity receptors while the second plateauing part of the curve denotes the low affinity receptors. A representative plot of bound against bound/free receptors in normal and CML PMNL using fNLPTNL is shown in Figures 5 and 6, respectively. All the normal and CML samples showed such linear plots.

The values of number of receptors/PMNL from normal subjects and CML patients are shown in Figure 7. The mean number of high affinity receptors/PMNL from granulocytes of 17 normal subjects was $12.18 \pm 0.72 \times 10^4$

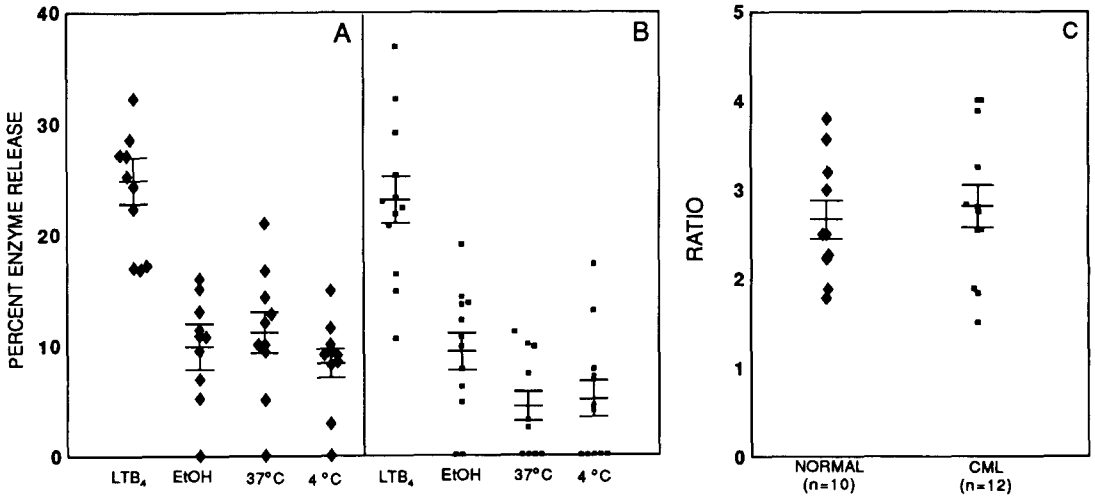

Fig. 4. Scattergram showing PER by cells stimulated with LTB_4 and under control conditions (EtOH, 37°, 4°C). Ordinate: PER: A: Normal PMNL. B: CML PMNL. C: Ratio of lysozyme released after stimulation with LTB_4 as compared to that under unstimulated conditions in normal and CML PMNL. Bars show mean ± SEM.

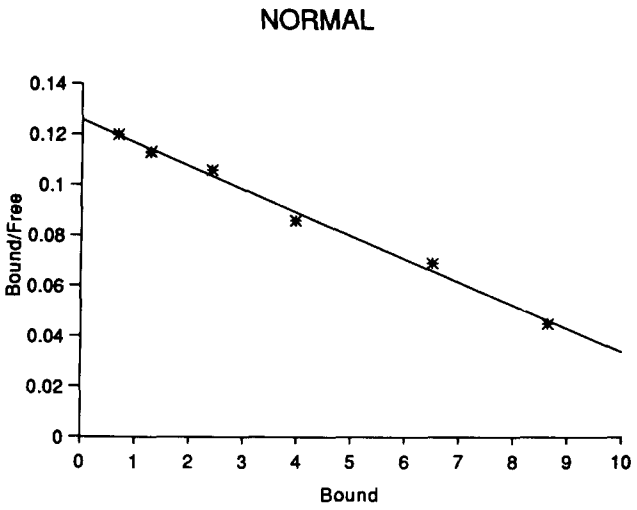

Fig. 5. Representative plot of bound vs. bound/free ^{125}I labelled fNLPNTL binding to normal PMNL.

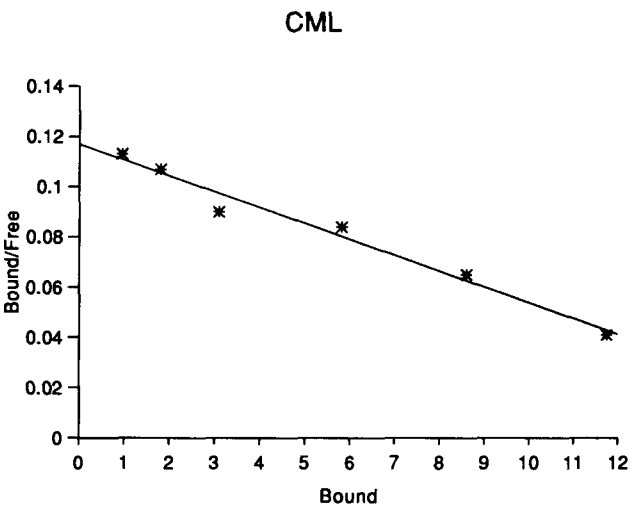

Fig. 6. Representative plot of bound vs. bound/free ^{125}I labelled fNLPNTL binding to CML PMNL.

and for that from 15 CML patients was $9.43 \pm 0.57 \times 10^4$M. The number of receptors per PMNL is significantly higher in normal PMNL as compared to CML PMNL ($P < 0.05$).

The values of affinity constants of fNLPNTL to its receptors on PMNL from normal subjects and CML patients are shown in Figure 8. The mean binding affinity for PMNL from 17 normal subjects was $8.51 \pm 0.51 \times 10^7 M^{-1}$ and the mean binding affinity for PMNL for 15 CML patients was $8.43 \pm 0.38 \times 10^7 M^{-1}$. There was no significant difference in the affinity constants of PMNL from normal and CML subjects.

The values of dissociation constants of fNLPNTL for its receptors on PMNL from normal subjects and CML patients are shown in Figure 9. The mean dissociation constants for PMNL from 17 normal subjects was $1.24 \pm 0.68 \times 10^{-8}$M and the mean dissociation constant from 15 CML patients was $1.19 \pm 0.66 \times 10^{-8}$M, respectively. The difference in the dissociation constants of PMNL from normal and CML subjects was not statistically significant.

DISCUSSION

Polymorphonuclear leukocytes are the first cells to reach the site of infection or tissue damage. This remote

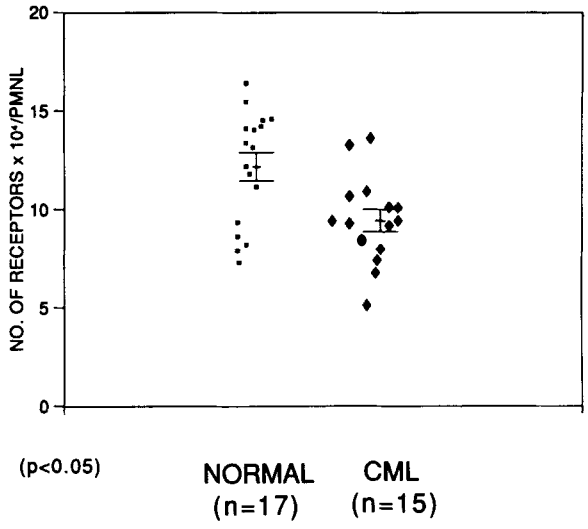

Fig. 7. Scattergram showing number of receptors/PMNL for fNLPNTL for normal and CML PMNL. Bars show mean ± SEM.

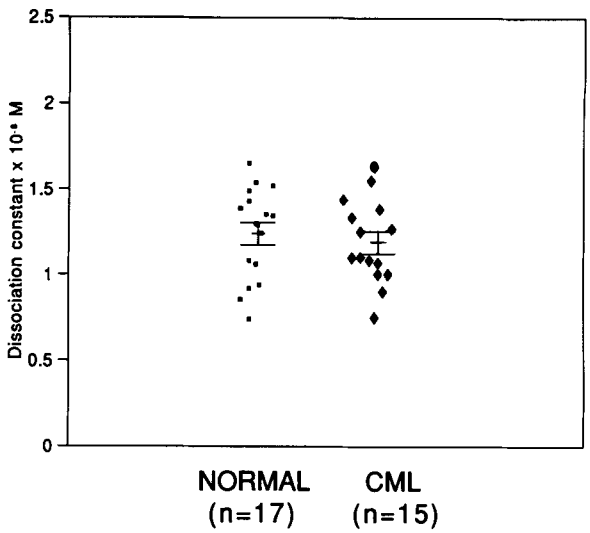

Fig. 9. Scattergram showing dissociation constants of fNLPNTL receptors for normal and CML PMNL. Bars show mean ± SEM.

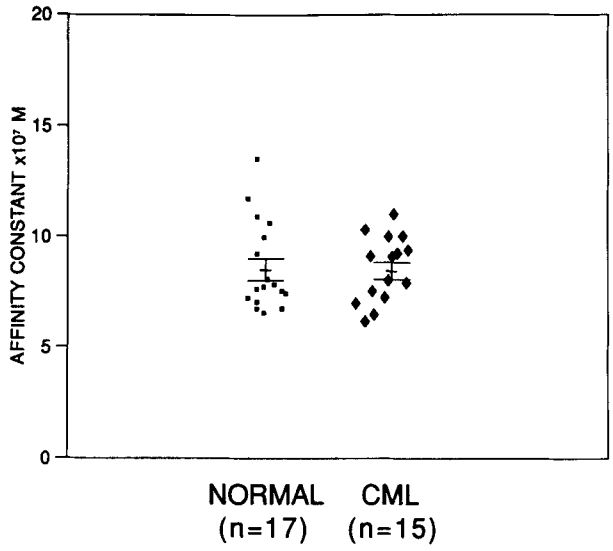

Fig. 8. Scattergram showing affinity constants of the fNLPNTL receptors for normal and CML PMNL. Bars show mean ± SEM.

sensing is made possible by the presence of chemoattractants at the inflamed site. Binding of chemoattractants to receptors on the surface of PMNL leads to a series of events like actin and tubulin polymerization, orientation of cells, chemotaxis, pinocytosis, phagocytosis, and stimulation of microbicidal pathways. The latter include respiratory burst resulting in the production of superoxide and other reactive oxygen species, and degranulation, which corresponds to the release of the lysosomal hydrolases [1,16]. Neutrophils have distinct plasma membrane receptors for individual chemoattractants and they act independent of each other [10]. These chemoattractants share their ability to stimulate lysosomal enzyme release and respiratory burst. Receptors of all chemoattractants are associated with pertusis toxin sensitive guanine nucleotide binding protein-G-protein [2,17,18].

Previous studies from our laboratory have shown defective chemotaxis, lower actin polymerization, and reduced pinocytosis in CML PMNL in response to fMLP [5,7,8]. The present studies were undertaken to see whether this lower response of CML PMNL to fMLP is a phenomenon encompassing all chemoattractants or is confined only to fMLP pathway. Our results show that release of lysozyme from the granules after stimulation with fMLP and FACC[8] was significantly lower in CML PMNL as compared to that in normal cells while there was no difference between the two after stimulation with either PAF or LTB_4.

The interaction of chemoattractants with membrane receptors involves the classic signal transduction pathway, namely, the G-protein induced activation of phospholipids [19]. fMLP and FACC[8] are peptide chemoattractants whereas PAF and LTB_4 are lipid chemoattractants. Rabbit neutrophils contain two inhibitory G proteins-G_{i2} and G_{i3} [20]. G_i proteins cause inhibition of adenylate cyclase and activation or inhibition of several ion channels. It is possible that the G_i protein coupling with peptide chemoattractant receptors is different from that with lipid chemoattractant receptors and the differential response seen in CML cells could be due to an alteration in the interaction of one of these. McLeish et al. [21] have reported that in neutrophil-like HL-60 cells, both fMLP and LTB_4 activate a common G_i protein but their effects on (^{32}p) ADP ribosylation by cholera toxin

of a 40-KDa protein are different. Such differential interaction of a common G_i protein with each receptor may cause different levels of stimulation in CML PMNL.

Defective degranulation has been reported in a variety of clinical conditions such as congenital specific granule deficiency, where the patients' neutrophils demonstrate abnormal chemotaxis and bacterial killing in vitro [22]. In Chediak-Higashi syndrome (CHS), the neutrophils are characterised by impaired chemotaxis, delayed degranulation, and diminished amounts of granule proteases which may be due to a defect in mechanisms regulating cytoplasmic microtubule (MT) assembly [22–25]. However, Perou and Kaplan [26] have shown that the lysosomes in CHS are capable of interacting with MT based motors and delayed degranulation is not due to altered MT elements.

Structure-activity correlations using a wide variety of chemotactic peptide analogs have been interpreted in terms of extended beta-sheet structure as the biologically active receptor-bound conformation of the peptide. fMLP has an extended beta sheet structure while its analogs such as $FACC^6$ or $FACC^8$ have folded beta turn conformations [27]. Studies using stereochemically constrained analogs suggest that beta turn solutions are indeed biologically more active. Sukumar et al. [27] have reported that formyl-methionine-1-aminocyclohexane-1-carboxylic acid-phenylalanine-Omethionine ($FACC^6$) is more active than for-met-leu-phe-OH in rabbit neutrophils. We, therefore, evaluated degranulation of normal PMNL and CML PMNL using $FACC^8$ to see whether a difference in the conformation has a role to play in stimulation of CML PMNL. Our results clearly show that both these chemoattractants exert a similar reduced effect. This may be due to the lower number of fMLP receptors on these cells, resulting in an alteration in the signal transduction mechanism associated with it.

The apparent molecular size of the formyl peptide receptor was originally reported to be varying from 50,000–70,000 [28]. The number of receptor molecules for fMLP on the surface of the human PMNL has been shown to range between 2,000 to 120,000 by various investigators [11,29,30]. The data regarding the exact number of receptors for fNLPNTL per human PMNL is contradictory. Sklar et al. [31] using fluoresceinated peptide (fNLPNTL) showed the number of receptors at 15°C to be 53,000 ± 13,000 per human PMNL with a k_d of 0.6 ± 0.2 nM. Their analyses, with fluoresceinated peptide, carried out at temperatures varying between 4°–37°C showed temperature dependent variations in values of association constant. Niedel et al. [11] using ^{125}I-fNLPNTL, however, showed that there are 120,000 binding sites per human PMNL with a k_a of $1 \times 10^8 M^{-1}$ at 24°C. The reason for the difference seen in the number of receptors per human PMNL by iodine labelling as against fluorescent labelling is yet to be explained.

In the present study, we have used ^{125}I-fNLPNTL and find the number of receptor molecules per PMNL from normal subjects are in general agreement with those reported by Niedel et al. [11]. The number of receptors per PMNL from CML patients was significantly lower ($P < 0.05$), whereas the association constant values (k_a) for PMNL from normal subjects and CML patients were comparable.

Equilibrium binding studies using radiolabelled n-formyl peptides to examine receptor affinity states in neutrophils have demonstrated the presence of both high and low affinity forms of the receptors. Lane and Snyderman [32] and Goetzl et al. [33] have shown the existence of two classes of binding sites. Binding of ^{125}I-fMLPK to the recombinant fMLP receptor expressed in Cos cells was found to exhibit high and low affinity receptor while Bt_2cAMP differentiated HL-60 cells displayed a single class of binding sites [34]. Niedel et al. [11] have reported that the hexapeptide fNLPNTL displays high affinity saturable receptors. In the present study a single linear graph was obtained in every sample studied, which represents only high affinity receptors.

Baker et al. [35] and Wysocki et al. [36] reported decreased binding of fMLP to PMN cells from CML patients at diagnosis and in remission. This subnormal binding increased by pretreating the cells with neuraminidase. Interactions of PMNL with their microenvironment are also altered in CML patients. The levels of GM-CSF, which regulates fMLP receptors on PMNL, are higher in CML PMNL than in normal PMNL [37]. Chemotaxis dysfunction in humans with juvenile periodontitis is also accompanied by diminished receptor-mediated binding of chemoattractant. The neutrophils exhibit fewer than normal cell surface receptors for fMLP [38].

The fMLP receptor number in the plasma membrane appears to be correlated with the magnitude of the response observed after ligand challenge [39]. Incubation of neutrophils with degranulation stimuli increases the availability of fMLP receptor on the surface twofold [40]. Zigmond et al. [41] also found a transient increase in receptor binding activity within the first minute after stimulating the rabbit neutrophils with fMLP. They attribute this increase to augmentation of the absolute number of receptors rather than increased affinity. In the present study, however, it is observed that both the number and degranulation are significantly reduced in CML PMNL.

Chemotaxis dysfunction in humans with juvenile periodontitis was accompanied by diminished receptor mediated binding of chemoattractant [40]. This can occur as a result of decreased modulation of the receptors or increased rate of receptor desensitization. Cytoskeleton has been shown to play a role in receptor modulation in PMNL plasma membrane [41]. We have reported lower actin polymerization after fMLP stimulation and decreased amounts of cytoplasmic actin in CML PMNL

[7,9,42]. The significantly lower release of lysozyme observed by us after treatment with fMLP and $FACC_8$ may be due to a combined effect of a lower number of fMLP receptors and alterations in the actin cytoskeleton.

ACKNOWLEDGMENTS

We are grateful to Prof. P. Balram, Molecular Biophysics Unit, Indian Institute of Science, Bangalore, India, for the generous gift of $FACC^8$.

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